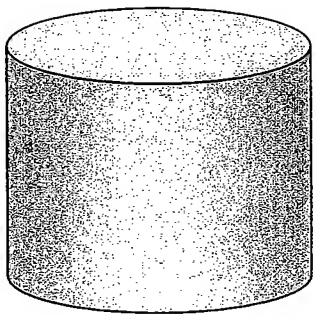
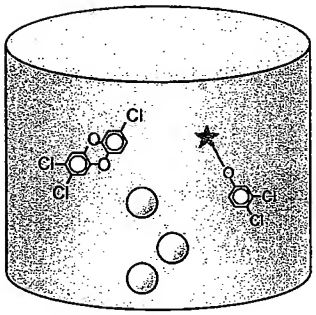


1 ml of 10 mM phosphate buffer solution (pH 8) containing 20-30% 1,4-dioxane

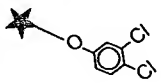
mix

①1 μl of 1 μM NBD-labeled dichlorophenol
②1 μl of test sample
③3 dioxin binding peptide beads
(①and ② are dissolved in 100% 1,4-dioxane solution)

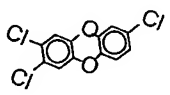


react (15 hr or more)

①



②



③

1. Record fluorescent microscope images of the beads.
2. Conduct tests using a sample of known concentration to create a calibration curve.
3. Determine the concentration of a test substance based on the calibration curve.

Fig. 1

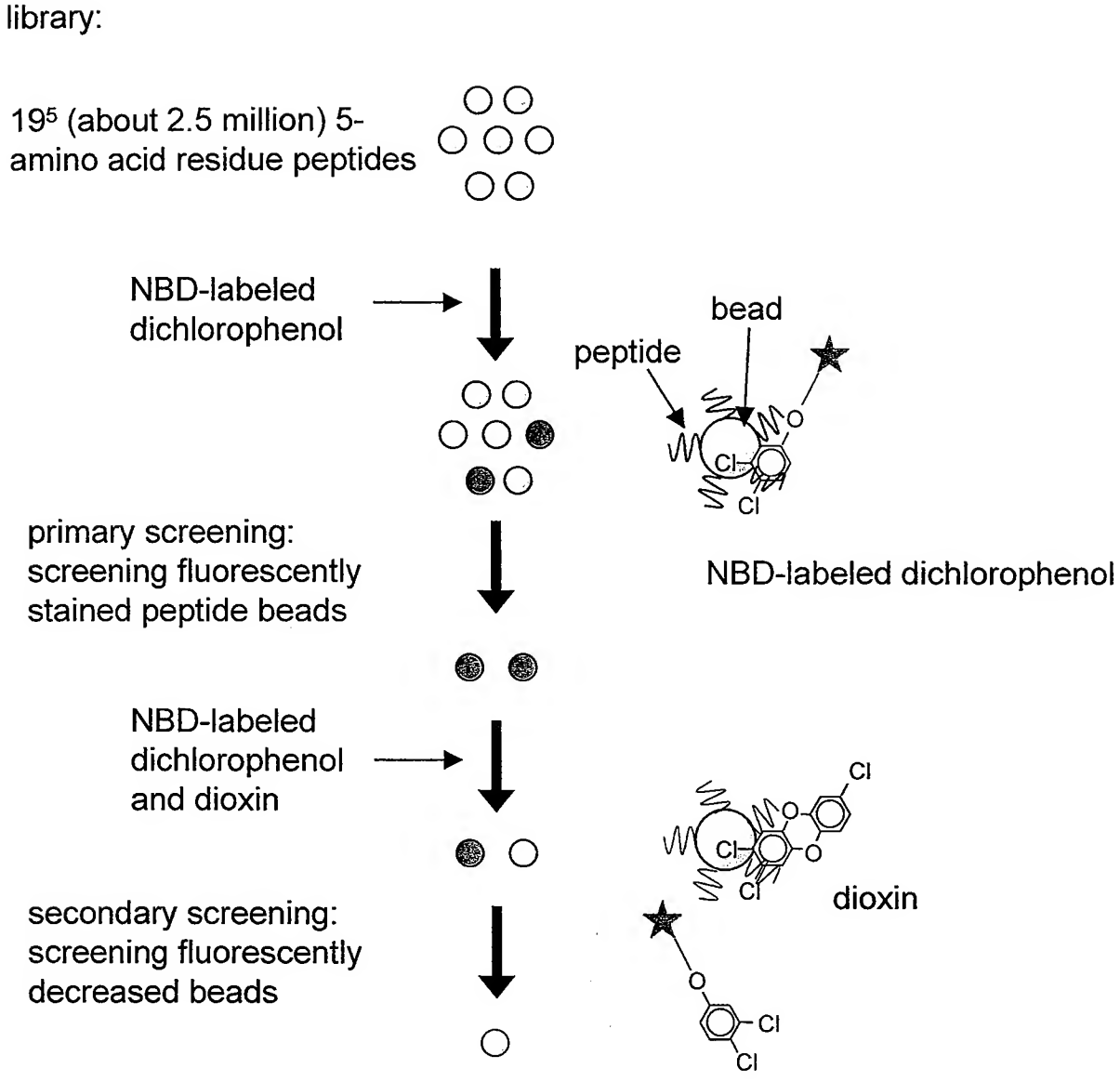
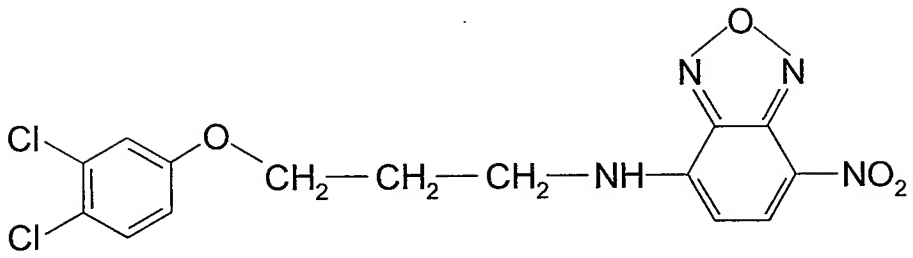
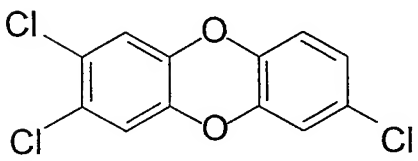

library:
19^5 (about 2.5 million) 5-amino acid residue peptides
NBD-labeled dichlorophenol
bead
peptide
Cl
Cl
primary screening: screening fluorescently stained peptide beads
NBD-labeled dichlorophenol
NBD-labeled dichlorophenol and dioxin
Cl
Cl
Cl
dioxin
secondary screening: screening fluorescently decreased beads
Cl
Cl

Fig. 2

NBD-labeled dichlorophenol



2,3,7-TriCDD



2,3,7,8-TeCDD

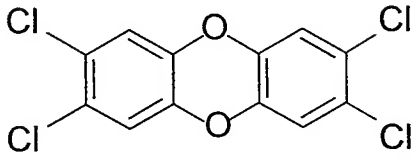


Fig. 3

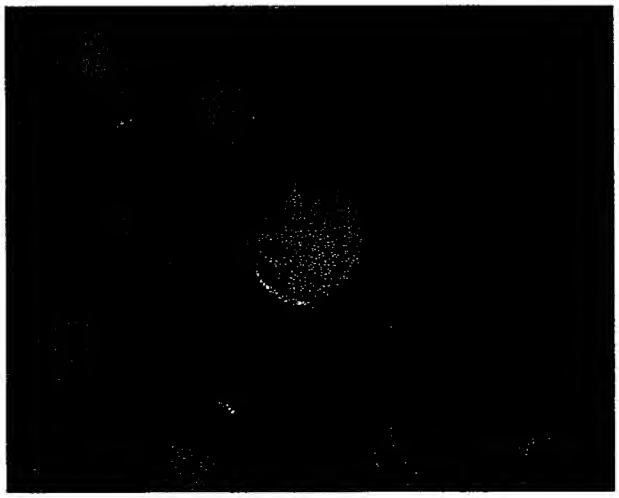
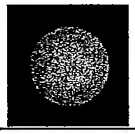
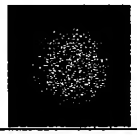
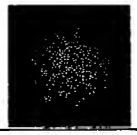


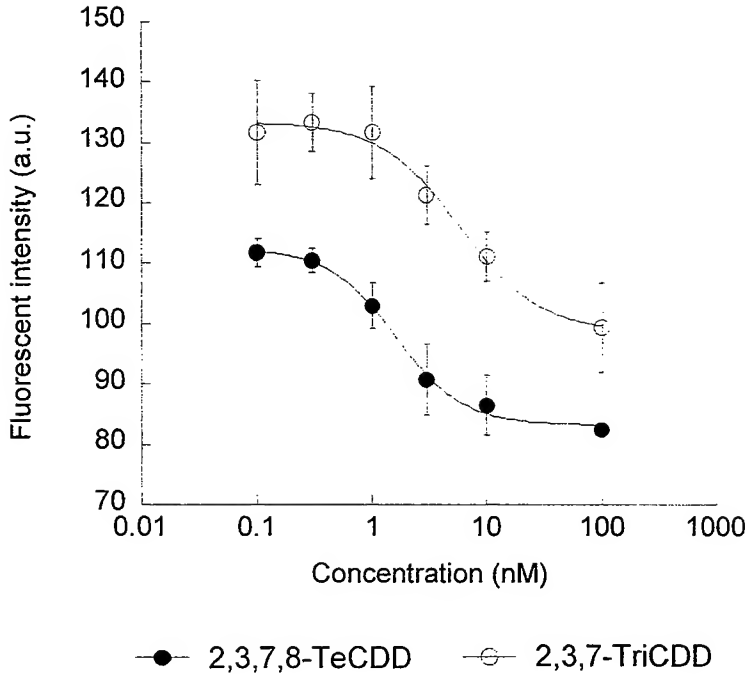
Fig. 4

	Before staining	After staining	After competition
Concentration of NBD-labeled dichlorophenol	0 nM	1 nM	1 nM
Concentration of competitive 2,3,7-TriCDD	0 nM	0 nM	10 nM (10 fold concentration)
DB2			
Reference (*)			

* Beads which were determined as not fluorescently stained in the primary screening

Fig. 5

(A)


150
140
130
120
110
100
90
80
70
Fluorescent intensity (a.u.)
0.01
0.1
1
10
100
1000
Concentration (nM)
2,3,7,8-TeCDD
2,3,7-TriCDD

(B)

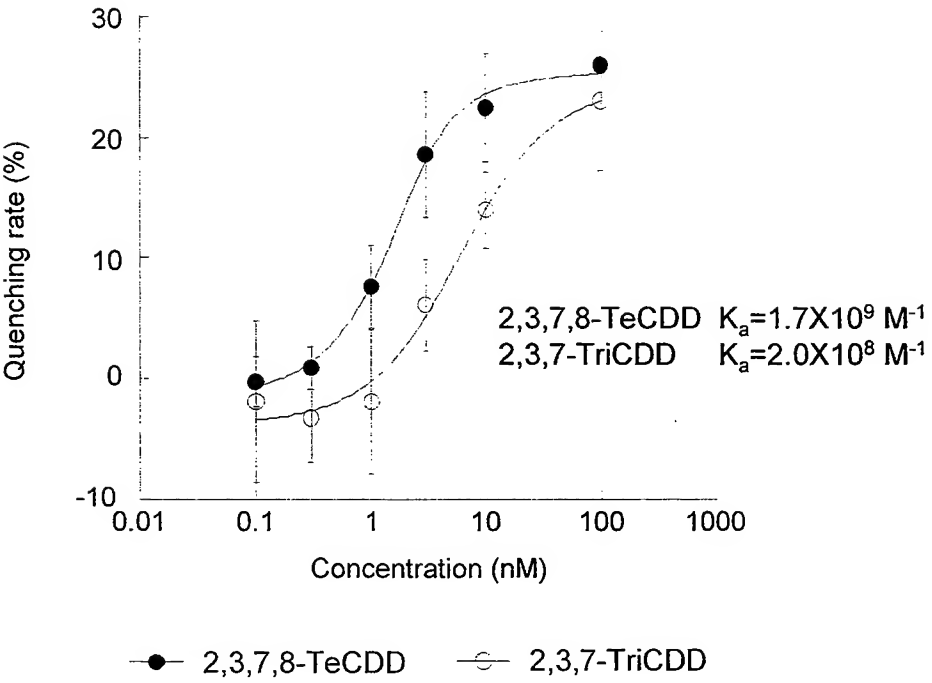

30
20
10
0
-10
Quenching rate (%)
0.01
0.1
1
10
100
1000
Concentration (nM)
2,3,7,8-TeCDD K_a=1.7X10^9 M^{-1}
2,3,7-TriCDD K_a=2.0X10^8 M^{-1}
2,3,7,8-TeCDD
2,3,7-TriCDD

Fig. 6

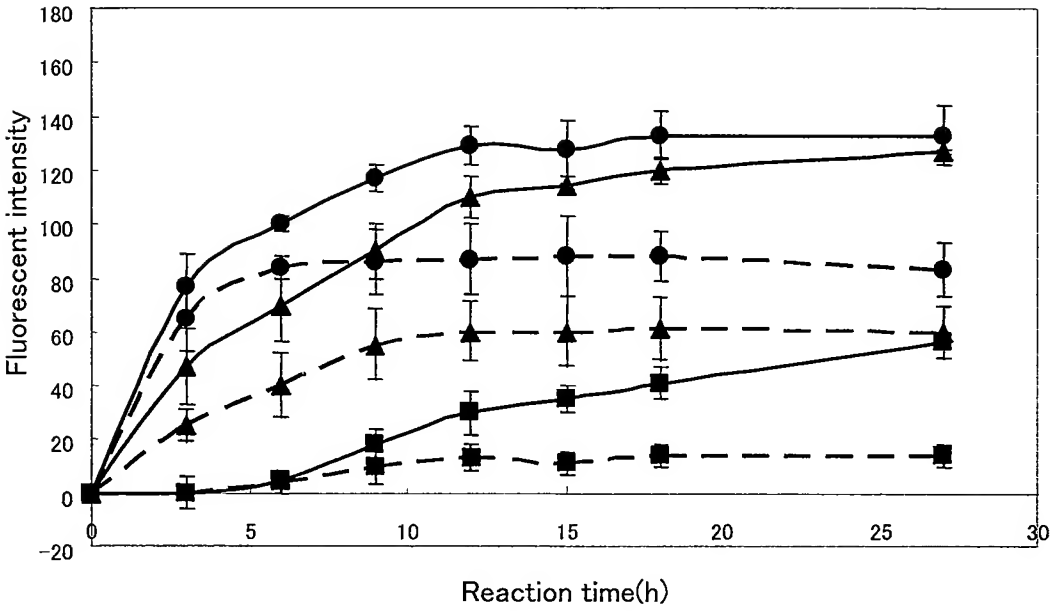
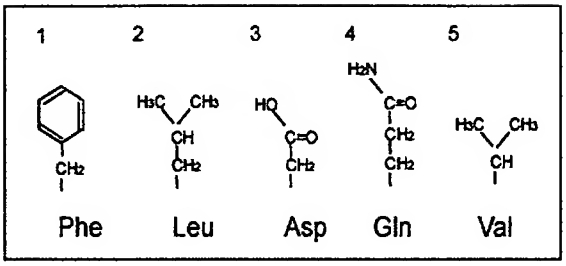

180
160
140
120
100
80
60
40
20
0
-20
Fluorescent intensity
0
5
10
15
20
25
30
Reaction time(h)

Fig. 7

structures of DB2 amino acid side chains



abbreviations

phenylalanine (Phe)
1-naphthylalanine (Nal(1))
cyclohexylalanine (Cha)
phenylglycine (phg)
valine (val)
alanine (Ala)
leucine (Leu)
isoleucine (Ile)
norvaline (Nva)
norleucine (Nle)
methionine (Met)
aspartic acid (Asp)
asparagine (Asn)
glutamic acid (Glu)
glutamine (Gln)

structures of substituted amino acid side chains

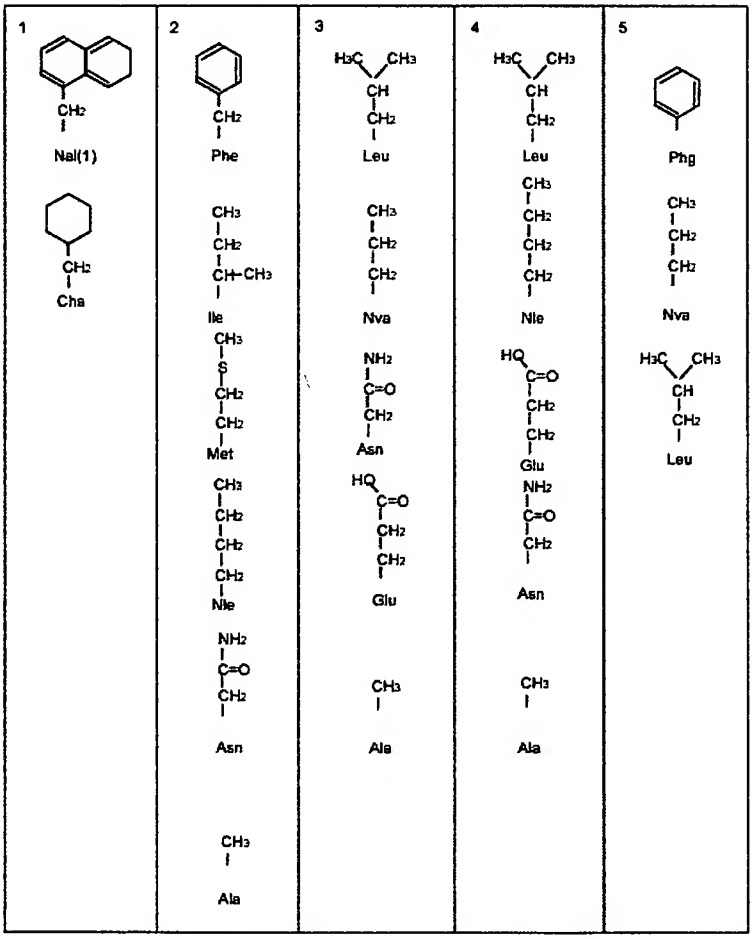


Fig. 8

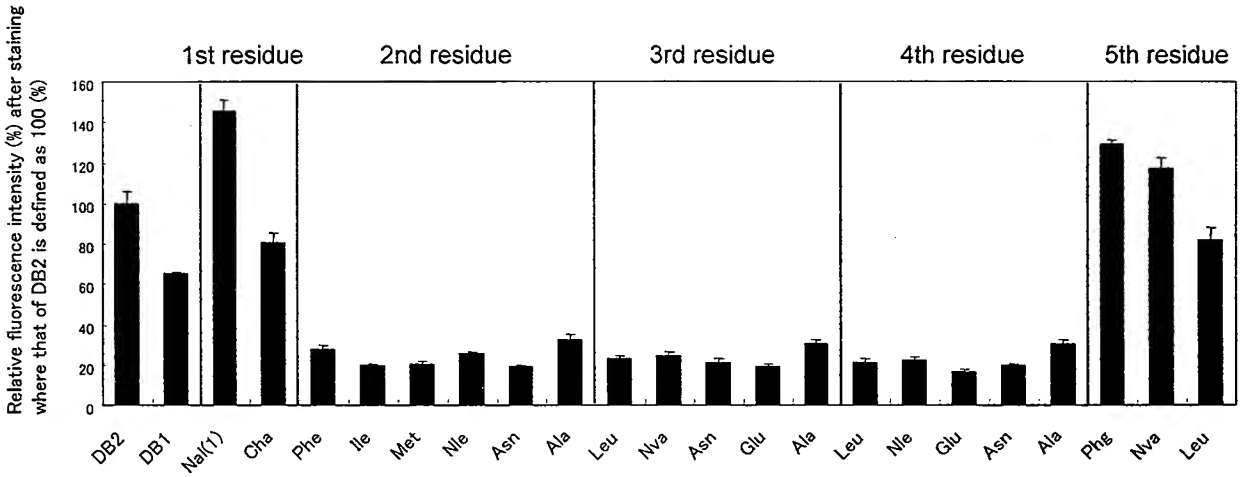

Relative fluorescence intensity (%) after staining where that of DB2 is defined as 100 (%)
1st residue
2nd residue
3rd residue
4th residue
5th residue
160
140
120
100
80
60
40
20
0
DB2
DB1
Nal(1)
Cha
Phe
Ile
Met
Nle
Asn
Ala
Leu
Nva
Asn
Glu
Ala
Leu
Nle
Glu
Asn
Ala
Phg
Nva
Leu

Fig. 9

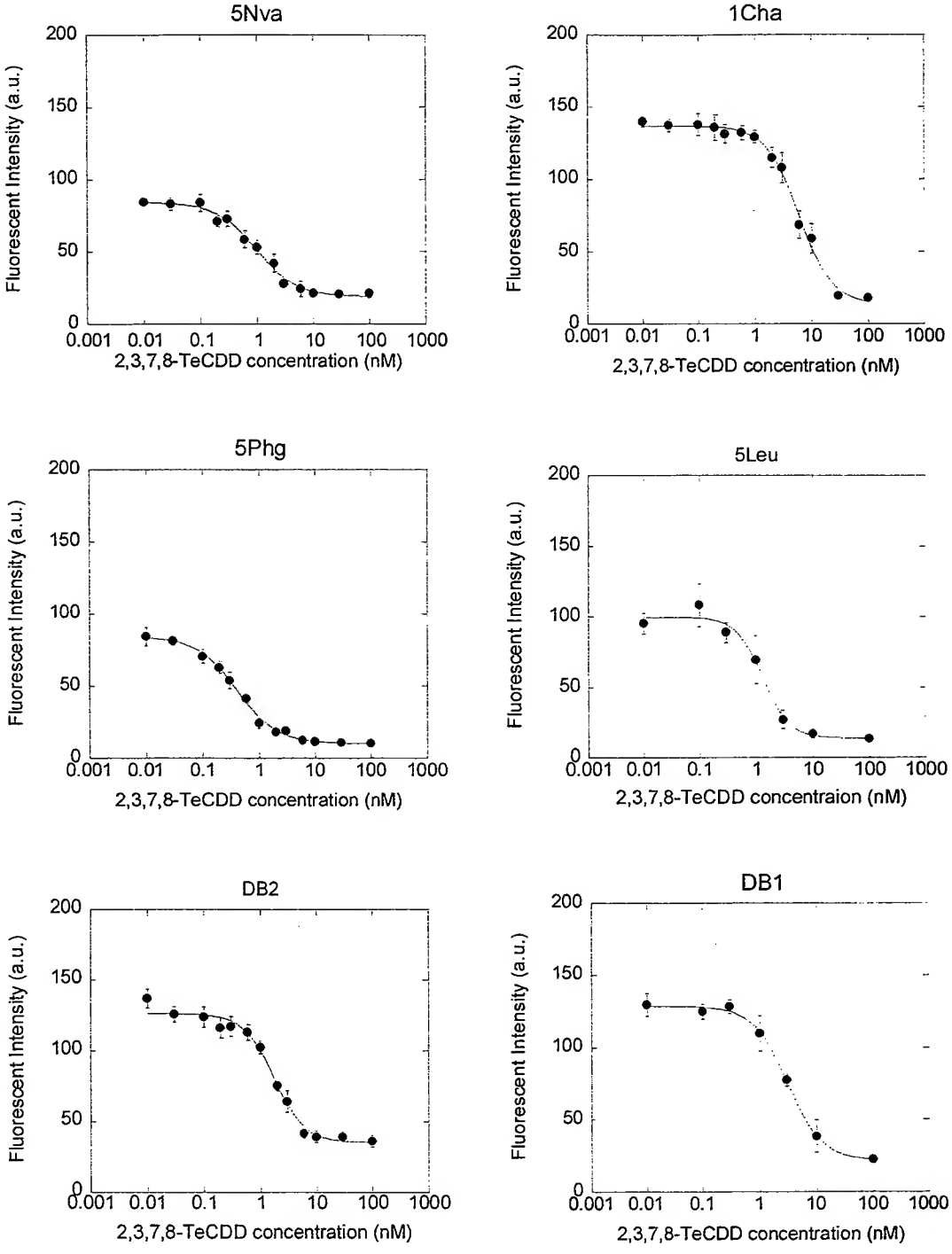
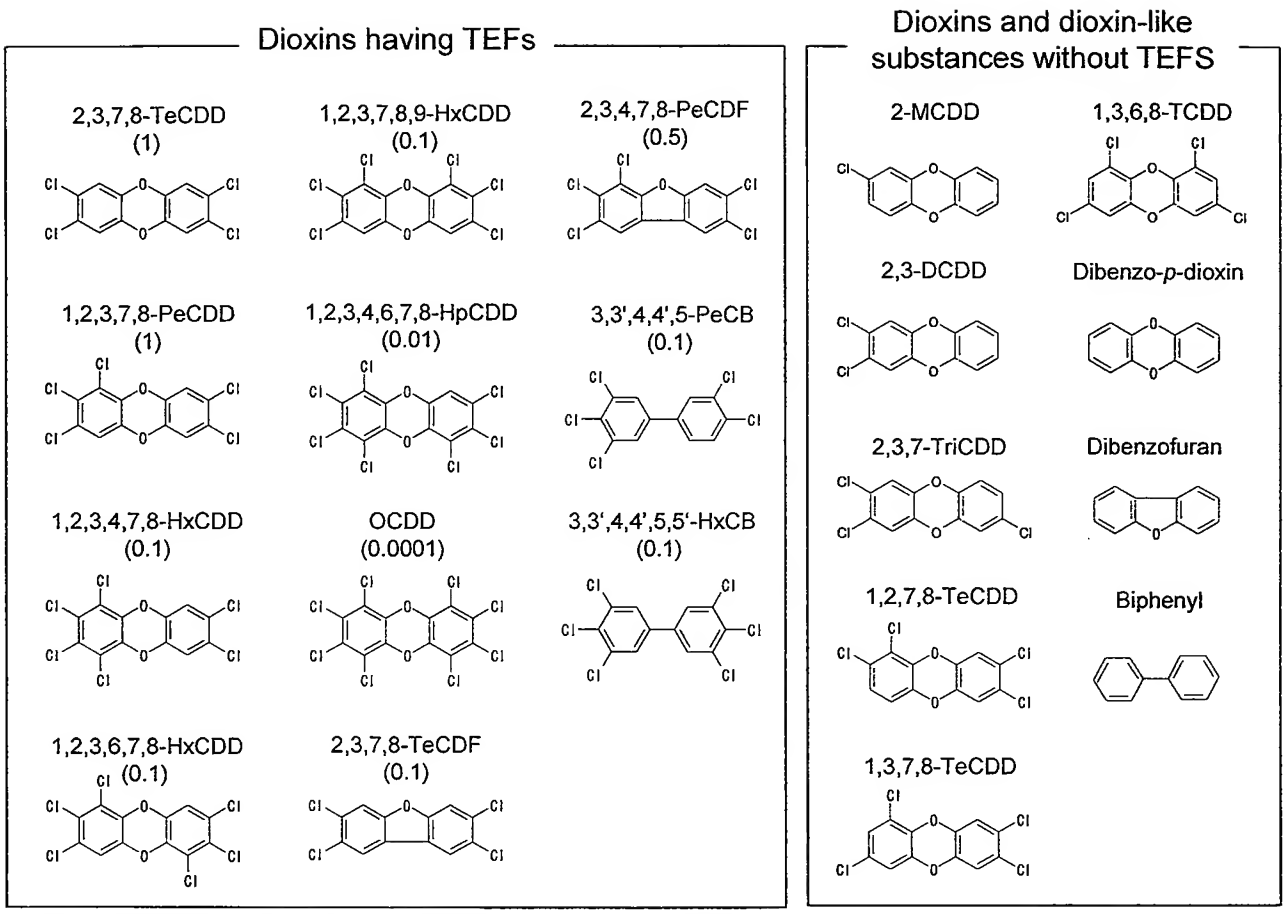

5Nva
1Cha
5Phg
5Leu
DB2
DB1
Fluorescent Intensity (a.u.)
2,3,7,8-TeCDD concentration (nM)
2,3,7,8-TeCDD concentraion (nM)
0
50
100
150
200
0.001
0.01
0.1
1
10
100
1000

Fig. 10



Note) Numbers in parentheses indicate toxic equivalency factors

Fig. 11

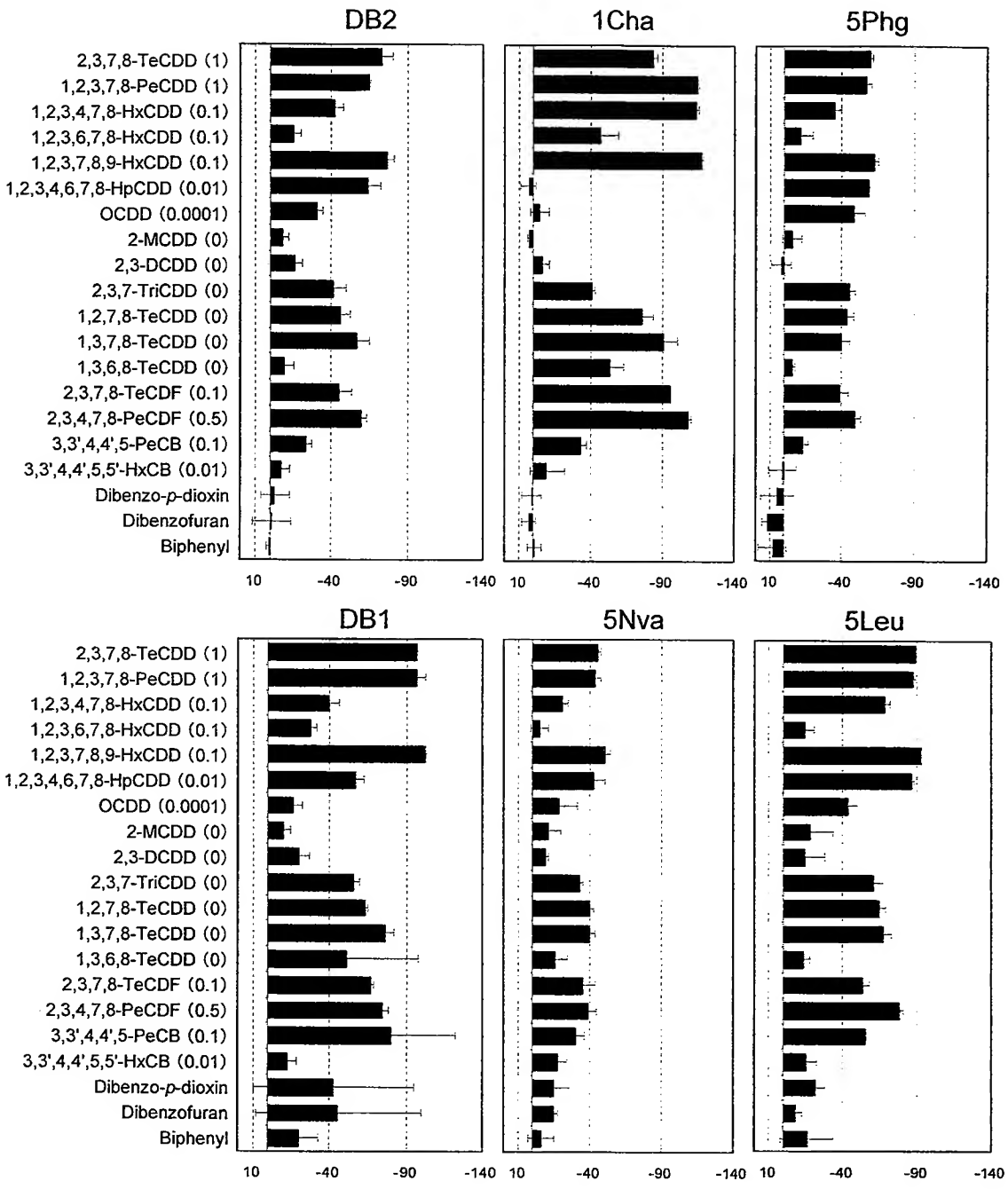

DB2
1Cha
5Phg
DB1
5Nva
5Leu
2,3,7,8-TeCDD (1)
1,2,3,7,8-PeCDD (1)
1,2,3,4,7,8-HxCDD (0.1)
1,2,3,6,7,8-HxCDD (0.1)
1,2,3,7,8,9-HxCDD (0.1)
1,2,3,4,6,7,8-HpCDD (0.01)
OCDD (0.0001)
2-MCDD (0)
2,3-DCDD (0)
2,3,7-TriCDD (0)
1,2,7,8-TeCDD (0)
1,3,7,8-TeCDD (0)
1,3,6,8-TeCDD (0)
2,3,7,8-TeCDF (0.1)
2,3,4,7,8-PeCDF (0.5)
3,3',4,4',5-PeCB (0.1)
3,3',4,4',5,5'-HxCB (0.01)
Dibenzo-p-dioxin
Dibenzofuran
Biphenyl
10
-40
-90
-140

Fig. 12